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PLASMA BIOCHEMISTRY VALUES OF GREEN TURTLES (*CHELONIA MYDAS*) WITH AND WITHOUT FIBROPAPILLOMAS IN THE HAWAIIAN ISLANDS

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**PLASMA BIOCHEMISTRY VALUES OF GREEN TURTLES (*CHELONIA MYDAS*)
WITH AND WITHOUT FIBROPAPILLOMAS IN THE HAWAIIAN ISLANDS**

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PREFACE

This report presents the results of research comparing the plasma biochemistry values of immature green turtles, *Chelonia mydas*, with and without fibropapilloma tumors, sampled from foraging pastures in the Hawaiian Islands. The work was conducted with funds supplied by the Southwest Fisheries Science Center (SWFSC) Honolulu Laboratory's Marine Turtle Research Program. The results of previous research conducted under contracts awarded to Dr. A. Alonso Aguirre can be found in SWFSC Administrative Reports H-92-07C, H-93-07C, H-93-11C, H-94-4C, H-94-09C, H-95-01C, and H-96-06C issued by the Honolulu Laboratory.

The incidence of life-threatening tumors on green turtles in the Hawaiian Islands has grown to epidemic proportions during the past decade. A similar situation exists among green turtles at certain sites in Florida, the Caribbean, and elsewhere worldwide. The cause of this disease, called fibropapillomatosis, remains unknown. However, a herpes virus, and recently a retrovirus, have been implicated. Death appears to be the usual result of the disease, although the impact to afflicted populations has not been fully assessed. The disease represents one more potentially significant threat to the survival of all green turtles. Recent findings in Florida have shown that the disease is now also occurring in increasing numbers in the loggerhead turtle, *Caretta caretta*.

The nature of fibropapillomatosis, along with its cause and mode of transmission, must be determined in order to develop a long-term management program of containment and prevention. The findings of the present report constitute progress in this direction which must be followed by additional research.

Because this report was prepared by independent investigators, its statements, findings, conclusions, and recommendations do not necessarily reflect the views of the National Marine Fisheries Service, NOAA.

George H. Balazs
Leader, Marine Turtle Research
Honolulu Laboratory
December 1996

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EXECUTIVE SUMMARY

Baseline information on blood biochemistry values is provided for two foraging aggregations of clinically healthy wild green turtles (*Chelonia mydas*) inhabiting nearshore environments in Kaneohe Bay, Island of Oahu, and the Kona Coast, Island of Hawaii. Mean reference values were compared to values obtained from green turtles with fibropapillomatosis (FP) at several stages of the disease collected at Kaneohe Bay. Statistically significant differences were identified for total protein values, blood urea nitrogen, and enzymatic values between healthy turtles and turtles with FP. Turtles with advanced FP were hypoproteinemic, hypoglobulinemic, hypoalbuminemic, hypoferremic, azotemic, and presented inverse calcium/phosphorus ratios, low cholesterol, and low triglyceride values, indicating the chronicity and severity of FP. In general, wild green turtle reference blood values were not comparable with other published studies available for *C. mydas* in other geographic areas. It is concluded that blood biochemistry values should be established for each foraging aggregation of a discrete population and by geographic area considering disease status, age, sex, and seasonal variations. The data generated from this research will be useful for the clinical assessment of green turtle populations and will contribute to the development of appropriate management and conservation strategies for this endangered species in the Hawaiian Islands.

INTRODUCTION

The green turtle (*Chelonia mydas*) in the Hawaiian Islands is a geographically isolated population protected under the United States Endangered Species Act and Wildlife Laws of the State of Hawaii. The population has demonstrated a gradual increase since 1978 (Balazs, 1991, 1996); however, a neoplastic disease known as fibropapillomatosis (FP) has reached epidemic proportions, threatening the species in the Hawaiian Islands and other parts of the world. A herpesvirus-like agent has been implicated as the primary etiology (Herbst et al., 1995) but other etiologic factors have been studied including infectious agents (Aguirre et al., 1994), a response to trematode ova (Dailey and Morris, 1994), environmental pollutants impairing the immune system (Aguirre et al., 1994), and chronic stress (Aguirre et al., 1995).

Although the establishment of baseline blood profiles for healthy wild sea turtles is deemed to be a priority for the conservation and management of the species, normal plasma biochemistry values have not been established for most populations, except for a recent study in the southern Bahamas (Bolten and Bjorndal, 1992). Plasma biochemistry values represent a valuable diagnostic tool for monitoring the health and condition of free-ranging marine turtles (Norton et al., 1990; Bolten and Bjorndal, 1992; Aguirre et al., 1995).

The objective of this study was to determine baseline blood biochemistry values of two discrete foraging aggregations of immature, free-ranging clinically healthy green turtles in two nearshore habitats in the Hawaiian Islands. In addition, their profiles were compared to a group of turtles with FP.

MATERIALS AND METHODS

During September/October 1991 to 1995, blood specimens were collected from a foraging aggregation of clinically healthy green turtles and turtles with FP in Kaneohe Bay (21°N, 157°W), Island of Oahu. Blood specimens were also collected from a foraging aggregation of clinically healthy, green turtles captured along the Kona Coast (19°N, 156°W) of the Island of Hawaii at Keawa Nui Bay and Kiholo Bay (see Balazs, in press).

Green turtles at Kaneohe Bay were captured by hand, alive and unharmed, using snorkeling equipment. Green turtles from the Kona Coast were also captured alive and unharmed by hand and by using a closely monitored tangle net. A blood specimen from each turtle was taken by venipuncture from the dorsal post-occipital sinuses (Owens and Ruiz, 1980) following manual restraint. Blood (3-10 ml) was collected using 21 gauge needles and 5-ml or 10-ml

syringes. Blood specimens were transferred into heparinized Vacutainer tubes (Becton, Dickinson & Co., Rutherford, New Jersey, USA). Plasma was separated by centrifugation at 2,000 rpm for 10 minutes, and split in two or more vials. Vials were stored on dry ice or in an ultrafreezer at -70°C until a 25-element biochemistry analysis was performed at SmithKline Beecham Clinical Laboratories (Honolulu, Hawaii and Van Nuys, California) on each specimen. Plasma biochemistry values were determined using an automated random access analyzer Olympus 5000 Series AU5061 (Olympus Corporation, Lake Success, New York, USA). This autoanalyzer demonstrated excellent precision when measuring biochemistry values for loggerhead turtles (*Caretta caretta*) (Bolten et al., 1992). Hemolyzed samples were discarded. The following plasma determinants were measured: total protein, albumin, globulin, total bilirubin, alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase (AKP), gamma glutamyl transpeptidase (GGT), lactic dehydrogenase (LDH), urea nitrogen, creatinine, uric acid, calcium, phosphorus, cholesterol, triglycerides, glucose, iron, sodium, potassium, and chloride.

Following blood collection, turtles were measured, tagged, and weighed according to techniques previously described (Balazs et al., 1987). All turtles were thoroughly examined for the presence of fibropapillomas and a description of their size, number, and location was recorded. Turtles were assigned a fibropapilloma severity score (FPS) on a scale of 0-4, with FPS = 4 being the most severe case, FPS = 3 being heavily affected, FPS = 2 moderately affected, and FPS = 1 lightly affected. Turtles without fibropapillomas were given a score of 0. Anatomic site influenced FPS when vision or ability to feed was impaired (Balazs, 1991).

Statistical analyses applying one-way ANOVA, Kruskal-Wallis tests, and Student's *t* tests were performed using the SAS software (Schlotzhauer and Littell, 1987). Differences were identified for mean values between the two clinically healthy turtle aggregations, and among healthy turtles and turtles with different FPS. Results were considered statistically significant for probabilities < 0.05 ($\alpha = 0.05$). Data were expressed as mean, standard deviation (SD), and range of values for each blood parameter.

RESULTS

Mean, SD, and range of straight carapace length (SCL), weight, and blood biochemistry values for 90 clinically healthy green turtles by collection site are summarized in Table 1. Similar data for 56 green turtles with fibropapillomatosis and classified by FPS 1 to 4, are presented for Kaneohe Bay (Table 2). Mean SCL and weights were significantly smaller for

clinically healthy turtles in both aggregations when compared to turtles with fibropapillomatosis. In addition, FP severity scores increased with size and weight (Fig. 1).

Plasma enzymatic values were significantly different between both healthy turtle aggregations. The Kona Coast group presented higher ASAT and LDH values, while the Kaneohe Bay healthy turtle group had higher ALAT and AKP values. ALAT decreased with fibropapilloma severity, however, ASAT, AKP and LDH demonstrated the opposite trend (Tables 1 and 2).

Plasma parameters for the two wild healthy aggregations were similar in general; however, significantly higher values of total protein and globulin were identified for the Kona Coast aggregation when compared to the Kaneohe Bay aggregation (Fig. 2). Differences were also identified for calcium (Ca) and phosphorus (P) levels for the healthy aggregations. Significantly higher Ca and lower P levels were detected in the Kona Coast turtles when compared to the healthy, wild Kaneohe Bay population. The Ca/P ratios for Kona Coast and Kaneohe Bay turtles were 2:1 and 1:1, respectively (Fig. 3).

Comparisons between clinically healthy turtles of both aggregations and turtles with FP yielded significant results. Hypoproteinemia and related protein deficiencies were evident for turtles with advanced FP. Pooled Ca/P ratios were reversed in turtles with advanced stages of the disease. Iron levels sharply fluctuated in turtles with FP at Kaneohe Bay, declining with the degree of fibropapilloma severity from 46 mcg/dL to 71 mcg (Fig. 4). Likewise, blood urea nitrogen (BUN) increased with disease severity, producing highly disproportionate BUN/creatinine ratios (Fig. 5). Statistically significant changes in cholesterol and triglyceride levels were observed when comparing clinically healthy turtles to turtles with FP in Kaneohe Bay. A decline of these mean values was evident in turtles with advanced FP.

DISCUSSION

Baseline information was provided for blood biochemistry values of two foraging aggregations of free-ranging green turtles inhabiting nearshore environments in the Hawaiian Islands. Mean reference values from these turtles were compared to values obtained from turtles affected with FP at several stages of severity of the disease.

Many plasma biochemistry parameters reported herein for wild Hawaiian turtles were not comparable with other published studies based on wild, maricultured, or captive reared green turtles (Holmes and McBean, 1964; Berkson, 1966; Dessauer, 1970; Bonnet, 1979; Frair and Shah, 1982; Norton et al., 1990). Total protein, total bilirubin, glucose, uric acid, calcium, and chloride; however, were comparable to ranges reported for healthy, juvenile

wild green turtles from southern Bahamas (Bolton and Bjorndal, 1992).

Recent research by Aguirre et al. (1995) demonstrated low blood cholesterol values, low triglyceride values, hypoproteinemia, hypoglobulinemia, hypoalbuminemia, and hypoferrremia in an experimental group of wild green turtles with severe fibropapillomatosis (FPS = 3) from Kaneohe Bay, Island of Oahu. Norton et al. (1990) presented comparable results indicating the severity and chronicity of fibropapillomatosis. The present study yielded similar results for the Kaneohe Bay aggregation. Decrease of enzymatic activity plasma levels identified herein provided evidence of a clinical disease with chronic course in turtles with FP.

Enzymatic differences between the two foraging aggregations of clinically healthy green turtles may be explained by their exposure to disease. Higher enzymatic values in the Kona Coast turtles may reflect the absence of FP having been found in this population. Evidence of chronic stress and immunosuppression in turtles with FP was previously described for wild green turtles in Kaneohe Bay (Aguirre et al., 1995).

Variables including age, sex, diet, and seasonal changes will influence blood parameters (Lutz and Dunbar-Cooper, 1987). Normal Ca/P ratios in sea turtles have not been established. Stamper and Whitaker (1994) noted improvement of Ca/P ratios following change in diet of captive sea turtles. Differences in Ca and P levels between the two clinically healthy aggregations could not be explained except for possible differences in their diet based on natural sea grasses and algae. Both minerals are important for normal physiological activity, and their regulation requires integration of other hormones including vitamin D, calcitonin, and parathormone. Decreases of Ca observed in turtles with FP may be related to hypoalbuminemia or a rapid alkalization process for FP turtles. Inverse Ca/P ratios have been reported for clinically normal loggerhead turtles (Stamper and Whitaker, 1994).

Azotemia was clearly observed in turtles with FP and may be explained by the neoplastic process. Decline of cholesterol and triglyceride levels observed in turtles with severe FP may relate to loss of body condition and starvation.

The large variance in some of the parameters outlined in this study may indicate effects of population variability and lack of repeatability of sampling. More precise estimates can be achieved by increasing sample size and repeating our measurements with the same individuals over time. Also, sampling at different locations with different disease conditions is recommended to establish normal values of healthy individuals. Further research is warranted to identify and characterize blood chemistry differences among healthy and diseased green turtle populations.

Also more studies are needed to provide insight into host susceptibility to FP in different populations as it relates to blood chemistry including protein, mineral deficiencies (i.e., Ca/P ratios), and enzymatic factors. The significant differences observed between foraging aggregations of Hawaiian *C. mydas* confirmed that baseline data should be collected by geographic area, discrete population, foraging aggregation, seasonal variability, and presence of FP and other related disease conditions. The data generated from this study may be useful for clinical assessment of health and disease of two foraging aggregations of wild green turtles on nearshore habitats in the Hawaiian Islands. In addition, this study will contribute to the development of appropriate management and conservation tools for wild Hawaiian *C. mydas*.

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Table 1.--Mean, standard deviation, and range of straight carapace length, weight, and plasma biochemistry values for clinically healthy green turtles (*Chelonia mydas*), Hawaiian Islands, 1991-95.

Variable	Kaneohe Bay, Oahu			Kiholo and Kona, Hawaii		
	n = 53			n = 37		
	Mean	±SD ^a	Range	Mean	±SD ^a	Range
Straight Carapace Length	45.3	4.8	37.4-55.2	47.7	7.2	38.5-67.8
Weight	14.1	4.7	7.7-25.4	17.8	6.6	9-26
Protein (g/dl)	4.2	0.6	2.9-5.6	5.0	0.7	3.5-6.7
Albumin (g/dl)	1.7	0.4	0.6-2.2	1.7	0.2	1.3-2.0
Globulin (g/dl)	2.7	0.5	1.8-4.0	3.3	0.6	1.9-4.7
Albumin/Globulin ratio	0.6	0.2	0.2-1.2	0.6	0.1	0.4-0.9
Total Bilirubin (mg/dl)	0.2	0.1	0.0-0.4	0.2	0.04	0.0-0.3
Direct Bilirubin (mg/dl)	0.05	0.1	0.0-0.5	0.02	0.05	0.0-0.2
Indirect Bilirubin (mg/dl)	0.2	0.1	0.0-0.4	0.2	0.06	0.0-0.2
ALAT ^b (U/I)	3.9	7.0	0.0-50.0	2.3	1.6	1-7
ASAT ^c (U/I)	158.4	41.5	1.0-270	215.3	100.9	47-491
Alkaline Phosphatase (U/I)	33.5	12.2	12-62	18.2	8.5	5-42
GGT ^d (U/I)	2.7	1.5	0.0-5.0	1.0	0.0	1.0
LDH ^e (U/I)	203.8	180.4	55-1286	316.7	165.0	67-769
Urea Nitrogen (BUN) (mg/dl)	5.2	14.1	0.0-64.0	6.1	4.4	1-19
Creatinine (mg/dl)	0.2	0.1	0.1-0.5	0.2	0.1	0.1-0.3
BUN/Creatinine Ratio	25.8	70.7	0.0-320.0	32.9	31.1	3-160
Uric Acid (mg/dl)	1.3	0.8	0.0-4.5	1.5	0.7	0.7-3.9
Calcium (mg/dl)	9.1	1.7	1.1-12.1	11.2	2.2	4.8-15.0
Phosphorus (mg/dl)	8.2	1.3	5.9-11.8	5.0	1.3	2.8-10.7
Cholesterol (mg/dl)	140.0	43.0	32-280	99.1	34.0	42-184
Triglycerides (mg/dl)	124.2	68.7	28-331	84.0	53.3	24-245
Glucose (mg/dl)	114.7	35.0	64-234	109.1	13.2	86-133
Iron (mcg/dl)	46.3	64.8	9-321	43.5	25.5	23-91
Sodium (meq/l)	158.0	4.0	146-170	154.0	4.8	145-164
Potassium (meq/l)	5.2	0.9	3.9-8.6	4.8	0.5	4-6
Chloride (meq/l)	115.2	5.7	103-130	114.9	6.2	102-134

^aStandard Deviation

^bAlanine Amino Transferase

^cAspartate Amino Transferase

^dGamma Glutamyl Transpeptidase

^eLactic Dehydrogenase

Table 2.--Mean, standard deviation, and range of straight carapace length, weight, and plasma biochemistry values by severity of fibropapillomas (GTFP) for green turtles (*Chelonia mydas*), Kaneohe Bay, Island of Oahu, Hawaii, 1991-95.

Plasma biochemistry	TS = 1			TS = 2			TS = 3			TS = 4	
	Mean	"SD" ^a	Range	Mean	"SD"	Range	Mean	"SD"	Range	Mean	Value
Straight Carapace Length	53.3	3.7	46.9-60.8	53.9	6.5	46.8-67.2	58.0	10.3	43.0-88.6	55.3	
Weight	21.9	6.0	17.7-33.6	24.4	8.8	16.0-43.1	25.2	10.7	10.5-43.6	-	
Protein (g/dl)	4.9	0.6	4.0-6.1	4.5	0.8	2.9-6.3	3.5	1.0	1.8-5.7	3.9	
Albumin (g/dl)	1.9	0.2	1.6-2.2	1.7	0.3	0.8-2.0	1.2	0.4	0.4-1.8	-	
Globulin (g/dl)	3.0	0.5	2.2-3.9	2.7	0.5	2.1-3.5	2.3	0.7	1.1-4.0	-	
Albumin/Globulin Ratio	0.6	0.1	0.5-0.8	0.6	0.1	0.4-0.9	0.5	0.1	0.3-0.9	-	
Total Bilirubin (mg/dl)	0.2	0.1	0.0-0.2	0.2	0.1	0.0-0.3	0.1	0.1	0.0-0.4	0.0	
Direct Bilirubin (mg/dl)	0.02	0.1	0.0-0.2	0.02	0.04	0.0-0.1	0.02	0.1	0.0-0.3	0.0	
Indirect Bilirubin (mg/dl)	0.2	0.1	0.0-0.2	0.2	0.04	0.1-0.3	0.2	0.1	0.0-0.4	-	
ALAT ^b (U/I)	3.8	5.8	1.0-22	2.2	2.4	0.0-9.0	2.1	4.1	0.0-21.0	8.0	
ASAT ^c (U/I)	152.7	12.4	134-171	148.2	39.6	94-260	168.2	47.2	88-295	193.0	
Alkaline Phosphatase (U/I)	41.2	12.1	17-61	25.2	9.0	1-39	24.3	8.0	10-48	21.0	
GGT ^d (U/I)	-	-	-	2.8	1.3	1-4	1.1	1.7	0.0-5.0	4.0	
LDH ^e (U/I)	226.2	67.4	146-403	198.4	90.2	89-418	179.7	122.8	79-599	220.0	
Urea Nitrogen (BUN) (mg/dl)	1.9	2.1	0.0-8.0	6.5	14.2	0.0-56.0	14.8	19.6	1.0-78	24.0	
Creatinine (mg/dl)	0.2	0.1	0.1-0.4	0.2	0.1	0.1-0.4	0.2	0.1	0.1-0.4	0.4	
BUN/Creatinine Ratio	16.0	21.6	0.0-80	54.5	141.7	0.0-560	75.1	89.5	3.0-290	60.0	
Uric Acid (mg/dl)	1.0	0.2	0.6-1.5	1.1	0.6	0.5-2.8	1.6	2.9	0.1-16.2	1.5	
Calcium (mg/dl)	9.9	0.7	8.3-11	9.0	1.4	6.6-12.0	7.5	1.7	2.9-10.9	8.3	
Phosphorus (mg/dl)	8.4	1.0	6.7-10.6	7.7	1.0	6.5-9.3	8.0	2.0	5.3-13.4	9.3	
Cholesterol (mg/dl)	143.2	31.2	93-208	139.4	43.3	71-223	106.8	38.7	26-181	77.0	
Triglycerides (mg/dl)	141.8	49.2	78-231	124.7	87.6	25-300	84.1	54.5	32-278	26.0	
Glucose (mg/dl)	115.6	23.2	84-174	120.6	19.0	80-157	111.9	23.4	83-166	121.0	
Iron (mcg/dl)	-	-	-	70.8	76.1	16-183	34.7	21.0	9-76	24.0	
Sodium (meq/l)	158.6	4.1	151-164	158.0	5.4	152-171	154.5	4.7	141-165	160.0	
Potassium (meq/l)	5.5	0.7	4.4-7.2	5.1	1.0	3.5-7.1	4.5	0.8	3.8-7.7	5.9	
Chloride (meq/l)	113.6	4.6	109-125	113.9	6.5	103-126	113.5	5.9	101-130	126.0	
Standard Deviation	^b Alanine Amino Transferase			^c Aspartate Amino Transferase			^d Gamma Glutamyl Transferase			^e Lactate Deshydrogenase	

^aStandard Deviation

^bAlanine Amino Transferase

^cAspartate Amino Transferase

^dGamma Glutamyl Transferase

^eLactate Deshydrogenase

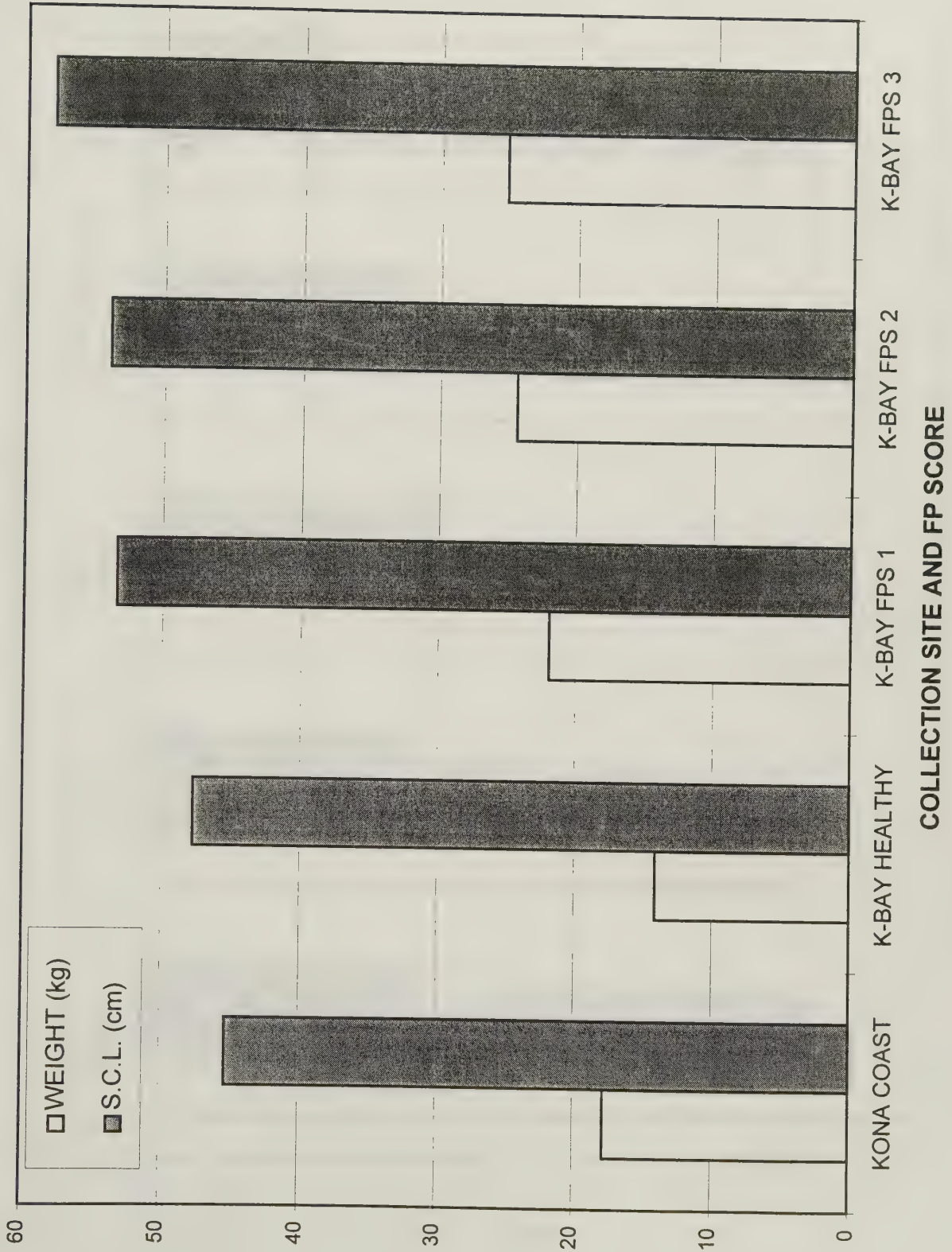
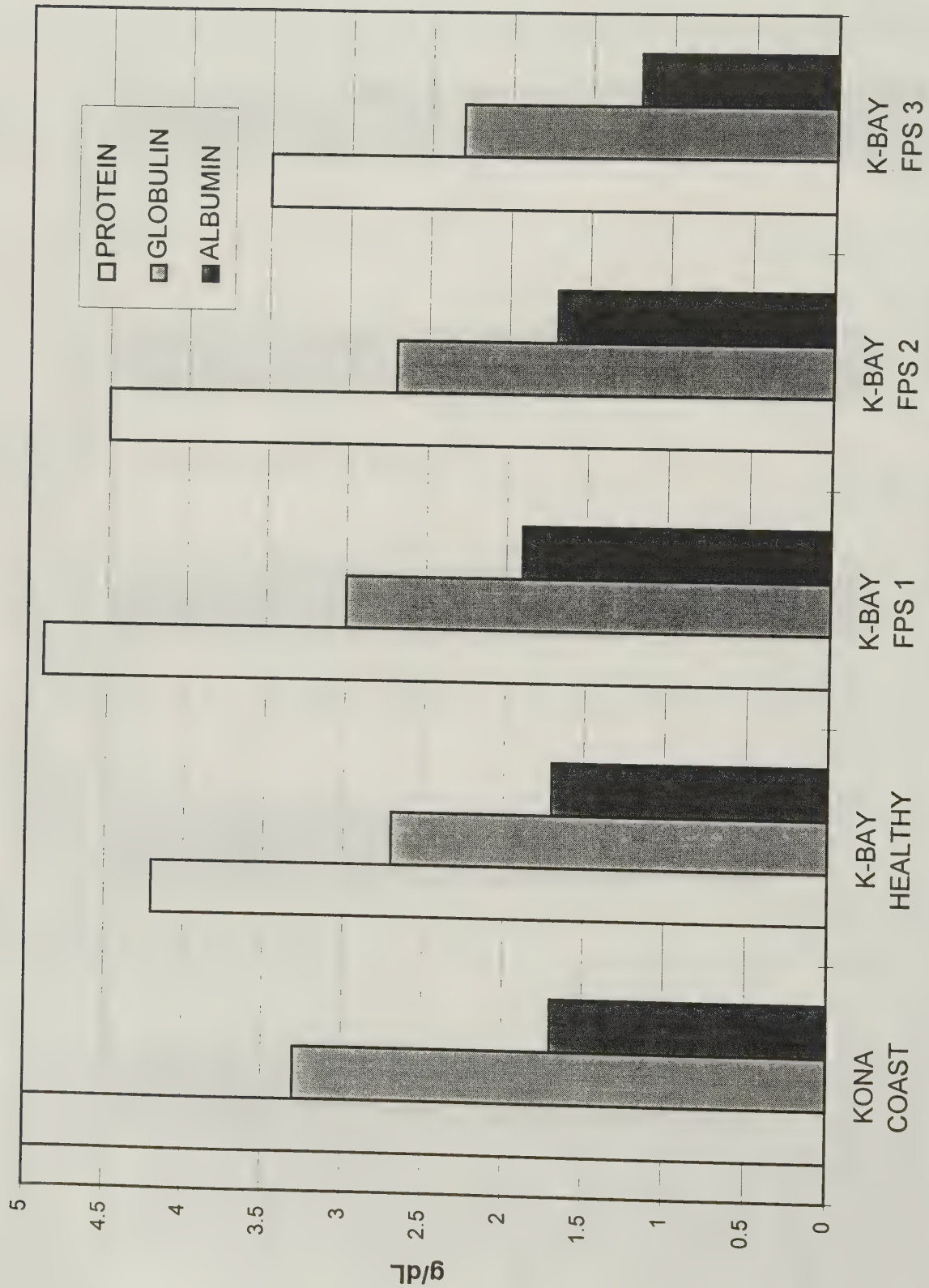


Figure 1. Mean straight carapace lengths (in centimeters) and weights (in kilograms) for clinically healthy green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (K-Bay), and turtles with fibropapillomatosis (FP) captured at Kaneohe Bay, 1991-95.



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Figure 2. Mean total plasma protein, globulin, and albumin values for clinically healthy green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (K-Bay), and turtles with fibropapillomatosis (FP) captured at Kaneohe Bay, 1991-95.

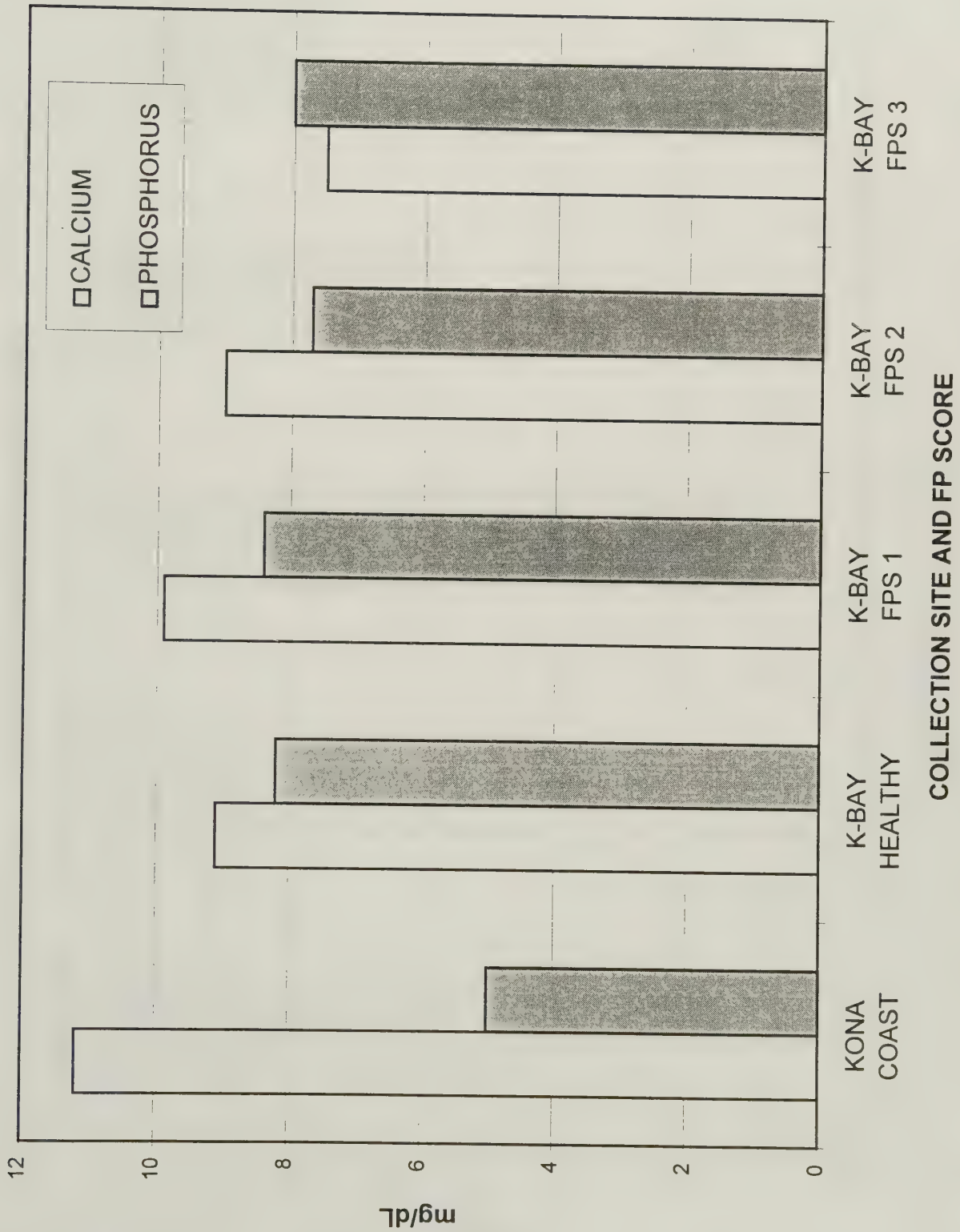


Figure 3. Mean calcium and phosphorus values for healthy green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (K-Bay) and turtles with fibropapillomatosis (FP) captured at Kaneohe Bay, 1991-95.

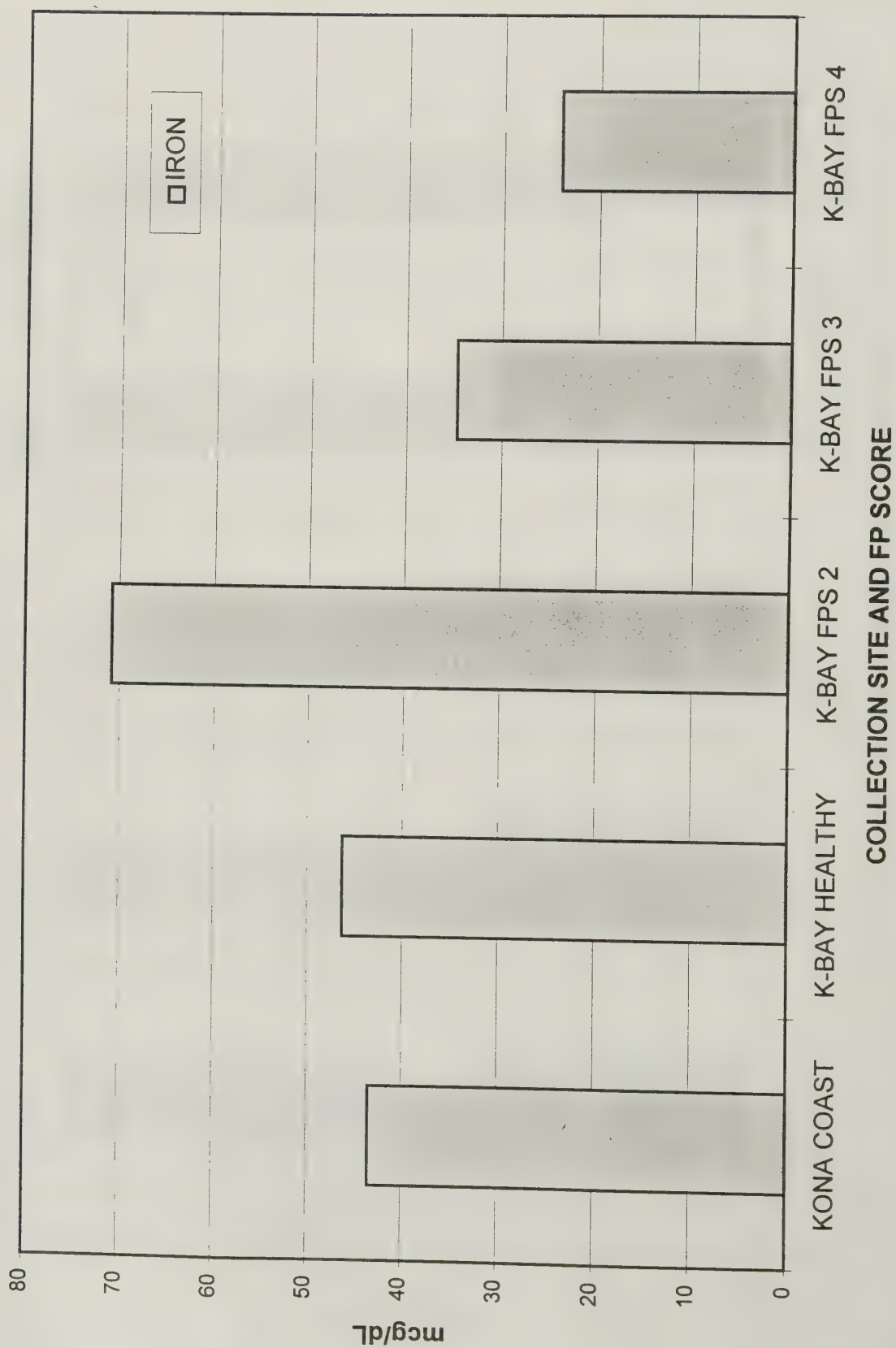


Figure 4. Mean iron levels for healthy green turtles (*Chelonia mydas*) captured at the Kona Coast and Kaneohe Bay (K-Bay) and turtles with fibropapillomatosis (FP) captured at Kaneohe Bay, 1991-95.

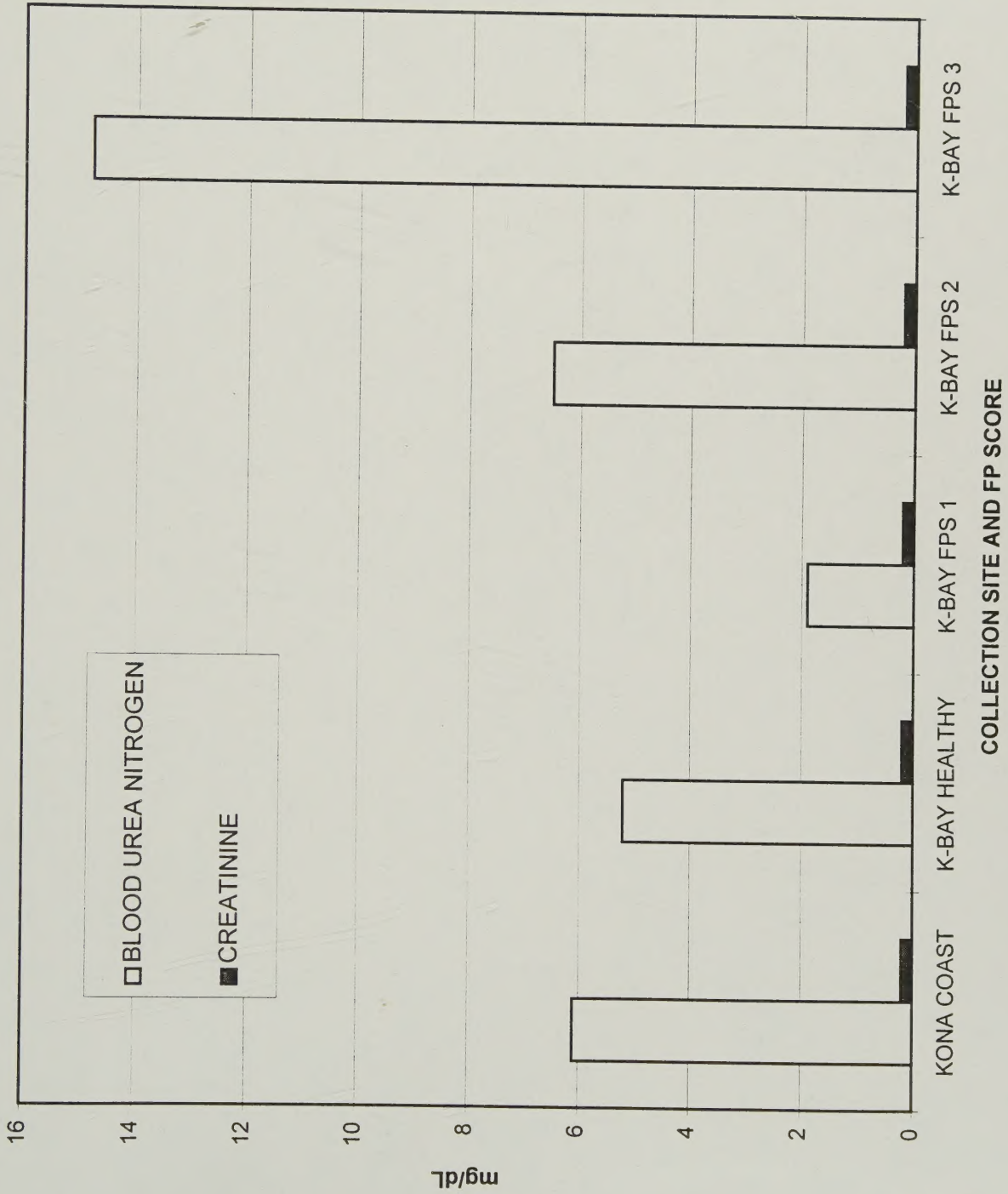


Figure 5. Mean blood urea nitrogen (BUN) levels and creatinine ratios for clinically healthy green turtles (*Chelonia mydas*) captured in the Kona at the Kona Coast and Kaneohe Bay (K-Bay), and turtles with fibropapillomatosis (FP) captured at Kaneohe Bay, 1991-1995.

The following table shows the results of the analysis of variance for the effect of the different treatments on the growth of the plants. The results are given in terms of the mean height of the plants in cm. The results are given in terms of the mean height of the plants in cm. The results are given in terms of the mean height of the plants in cm.



Figure 1. Mean height of plants in cm for different treatments. The results are given in terms of the mean height of the plants in cm. The results are given in terms of the mean height of the plants in cm.

